Blurring the boundaries between cereal crops and model plants

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Blurring the boundaries between cereal crops and model plants

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Summary

The cereal crops rice (*Oryza sativa*), maize (*Zea mays* ssp. *mays*) and wheat (*Triticum aestivum*) provide half of the food eaten by humankind. However, understanding their biology has proved challenging due to their large size, long lifecycle and large genomes. The model plant *Arabidopsis thaliana* avoids these practical problems and has provided fundamental understanding of plant biology, however not all of this knowledge is directly transferrable to cereals. Recent developments in gene editing, speed breeding and genome assembly techniques mean that the challenges associated with working with the major cereal crops can be overcome. Resources such as mutant collections and genome sequences are now available for these crops, making them attractive experimental systems with which to make discoveries that are directly applicable to increasing crop production.

I. Introduction

Across the biological sciences, model species have played major roles in improving our understanding of the fundamental processes that govern life. Decades of intensive study of the model plant *Arabidopsis thaliana* have produced insights into plant development and responses to the environment, particularly at the molecular level. However, it has long been recognised that one model species is not sufficient to represent the diversity of plants. Since the nineteenth century, when Mendel discovered the fundamental laws of inheritance using peas (*Pisum sativum*), researchers have studied many different plant model systems, from legumes through to tomato (*Solanum lycopersicum*), petunia (*Petunia hybrid*) and antirrhinum (*Antirrhinum majus*). Amongst the cereals, maize (*Zea mays* ssp. *mays*) has served as a genetic model system for almost a century, and its use in scientific research led to seminal discoveries about mobile DNA elements and epigenetics (reviewed in Nannas & Dawe (2015)). The sequencing of the Arabidopsis and rice (*Oryza sativa*) genomes almost 20 years ago opened up new possibilities for investigations into molecular genetics, with these species subsequently dominating research. However, we can now generate comprehensive genome assemblies even for highly complex plant genomes. This represents a turning point that could confer equal standing to multiple species for use in studies to understand the molecular mechanisms underlying plant biology and crop production. Here I will compare the features of model species that make them powerful research tools and outline recent developments that make the most widely grown cereal crops

– rice, maize and wheat (*Triticum aestivum*) – tractable experimental systems for molecular biology in their own right, thus blurring the boundaries between model species and cereal crops.

II. What defines a model plant?

Model species are extensively studied with the aim of understanding particular biological processes and the expectation that this will provide insight into other species. Model plant species have practical characteristics: small size, ease of growth, high fecundity, short generation time, small genome and amenability to genetic manipulation, including crossing, mutagenesis and gene modification. Recently, Chang *et al.* (2016) suggested that such practicalities are not the only reasons why model systems become widespread. As more scientists adopt the model species, the availability of simple and reliable methods for lab protocols such as DNA extraction, protein purification and transformation can influence its uptake. Furthermore, as a community of users develops, the availability of resources such as genetic stocks held in germplasm centres, annotated genomes and databases may boost its uptake.

III. Models for cereal crops

The three most widely grown cereal crops (maize, rice and wheat) provide *c.* 50% of the calories consumed by humankind (Alexandratos & Bruinsma, 2012). With an ever-increasing population and the challenges associated with climate change, we face an urgent need to understand the biology of these cereals to meet the growing demand for food, feed and fuel. Unfortunately, barriers such as their large genomes, long generation times and large sizes mean that cereals have proved difficult to work with.

Instead, from the 1980s onwards, Arabidopsis has been widely used as a model species with which to improve our understanding of plant biology. The study of Arabidopsis has enabled us to gain a fundamental understanding of many plant-specific processes, and this information has been used to inform the study of these same processes in cereal crops. Of the 41 682 papers published from 1965 to 2015 on Arabidopsis with one or more citations, 37% were cited by a paper principally focussed on a species other than Arabidopsis (Provart *et al.*, 2016), showing that Arabidopsis research has been used by those working on other species. However, certain processes which are highly relevant to crop production are not present in Arabidopsis (e.g. mycorrhization), and there are processes for which knowledge from Arabidopsis cannot simply be extrapolated (e.g. starch metabolism; Smith, 2012). Furthermore, although it has been shown that similar gene families are involved in regulating traits in Arabidopsis and cereals, the individual family members involved and the network of connections may be quite different, as has been shown for flowering (Hill & Li, 2016) and senescence (Borrill *et al.*, 2019a). Therefore, to fully understand cereal biology, research is required in cereal species themselves.

Several monocot models for crop species have been proposed to overcome some of the limitations of Arabidopsis research and improve our understanding of cereal biology. *Setaria viridis* was proposed as a model for maize and *Brachypodium distachyon* as a model for wheat due to their close evolutionary relationships and model plant characteristics (Brutnell, 2015; Table 1). However, when these species were proposed, molecular work was already being carried out in rice which demonstrated the power of studying a crop species directly to apply discoveries in the field. Rapid developments in technology, building in part upon approaches developed in rice, meant that wheat and maize could be studied at the molecular level in their own right. Therefore, the use of monocot model species as stepping stones to maize and wheat was not widely adopted.

IV. Recent developments redefine model plants

The availability of genome sequences lays the foundation for molecular biology work. The advent of low-cost next generation sequencing, long-read technology, improved assembly and scaffolding methods mean that having a small genome size is no longer a key consideration when looking to produce a reference genome sequence. For the past 10 years, a high-quality genome sequence for rice (International Rice Genome Sequencing Project, 2005) and a draft genome sequence for maize (Schnable *et al.*, 2009) have been available. Recently, long-read technologies and optical mapping improved the maize genome sequence (Jiao *et al.*, 2017) and a chromosome-level assembly for the 16 Gb hexaploid genome of wheat was published (IWGSC *et al.*, 2018). The release of these high-quality genome sequences indicates that genome size no longer presents a technological barrier, although the cost of sequencing large genomes remains high.

The development of gene editing has also influenced our ability to study gene function in a range of plant species. Previously, the large-scale mutant or insertion line collections in established model species such as Arabidopsis and rice (reviewed in Holland & Jez, 2018; Hong *et al.*, 2019) gave researchers working on these plants a major advantage in characterising gene function. However, CRISPR-Cas9 has been shown to function in all three major cereal crops and can be used to produce transgene-free genome-edited plants that could be readily commercialised (reviewed in Zhu *et al.* (2017)). CRISPR-Cas9 is conventionally used to induce small deletions within genes to cause frame-shift or knock-outs but can be used to carry out a whole range of more complex editing such as specific base editing or epigenetic modification (reviewed in Adli (2018)). These methods are now being applied in cereals (Li *et al.*, 2018).

Developments have also been made to shorten generation times and reduce plant size (Fig. 1). Cereal breeders have been accelerating generation times for decades by stressing plants in small pots. This method has been successfully applied in large scale commercial and public breeding programmes, for example at the International Rice Research Institute (Collard *et al.*, 2017). Manipulating environmental conditions has also enabled 'speed breeding', which involves growing plants under longer day length and harvesting seeds before they are fully mature; this greatly accelerates wheat generation times (Watson *et al.*, 2018). An alternative approach taken to shorten cereal generation time is to select varieties with extremely rapid lifecycles, including wheat 'Apogee' (25 d) (Bugbee & Koerner, 1997), Fast-Flowering

Table 1 Characteristics of model species used for cereal crop improvement.

		Arabidopsis thaliana	*Brachpodium distachyon*	*Setaria viridis*	*Zea mays* ssp. *mays*	*Oryza sativa* ssp. *japonica*	*Triticum aestivum*
Classical traits	Small	✓	✓	✓	✗ (only rapid lifecycle varieties)	✗ (only rapid lifecycle varieties)	✗ (only rapid lifecycle varieties)
	Fast lifecycle (months)	✓ (*c.* 2)[a]	✓ (*c.* 2)[b]	✓ (*c.* 2)[b]	✗ (6–12)[a] (*c.* 2 months for rapid lifecycle varieties)[c]	✗ (*c.* 4)[a] (*c.* 2 months for rapid lifecycle varieties)[d]	✗ (4–6)[e] (*c.* 2 months for rapid lifecycle varieties or speed breeding) [e,f]
	Large number of seeds	✓	✓	✓	✓	✓	✓
	Easy to cross and mutate	✓	✓	✓	✓	✓	✓
	Small genome size (Mb)[I]	✓ (125)	✓ (355)	✓ (396)[g]	✗ (*c.* 2400)	✓ (389)[h]	✗ (*c.* 15 600)[i]
	Diploid genome	✓	✓	✓	✓	✓	✗
	Potential to manipulate gene function	✓	✓	✓	✓	✓	✓
Derived traits	Standardised protocols	✓ TAIR	✓ JGI	✓[j]	✓ bio-protocol.org	✓[k]	✓ wheat-training.com
	Genome assembly	✓	✓	✓	✓	✓	✓
	Expression atlas[II]	✓ Arabidopsis eFP	✓ gene2function.de	✓ Phytozome	✓ Maize eFP	✓ Rice eFP RiceXPro	✓ Wheat eFP wheat-expression.com
	Mutant collections[II]	T-DNA[l], transposon[m] TAIR	T-DNA[n] JGI	Not available	Transposon[o] MaizeGDB	t-DNA,[p] retrotransposon,[q] fast neutron[r] RiceGE	EMS mutation[s] Wheat TILLING

[I]From EnsemblPlants release 43. April 2019 (https://plants.ensembl.org/) unless otherwise noted.
[II]For full details see species specific reviews: Arabidopsis (Holland & Jez, 2018), *Brachypodium distachyon* (Scholthof *et al*., 2018), *Setaria viridis* (Huang *et al*., 2016), rice (Hong *et al*., 2019), wheat (Borrill *et al*., 2019b) and maize (Portwood *et al*., 2018). Example indexed, searchable, archived mutant collections are shown.
[a]Chang *et al*. (2016); [b]Brutnell (2015); [c]McCaw *et al*. (2016); [d]Hu *et al*. (2018); [e]Watson *et al*. (2018); [f]Bugbee and Koerner (1997); [g]From Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html#); [h]International Rice Genome Sequencing Project (2005); [i]IWGSC *et al*. (2018); [j]Reviewed in Huang *et al*. (2016); [k]see Yang (2013); [l]Sessions *et al*. (2002), Alonso *et al*. (2003), Kleinboelting *et al*. (2012); [m]Parinov *et al*. (1999), Kuromori *et al*. (2004); [n]Hsia *et al*. (2017); [o]Settles *et al*. (2007), Williams-Carrier *et al*. (2010); [p]Zhang *et al*. (2006); [q]Miyao *et al*. (2003); [r]Li *et al*. (2017); [s]Krasileva *et al*. (2017).

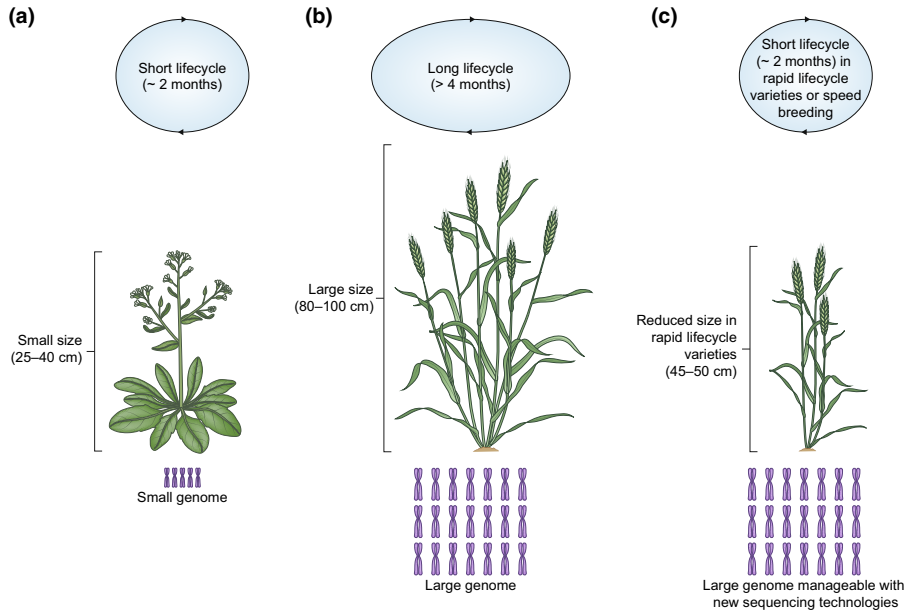

Fig. 1 Recent developments bypass traditional model species requirements. (a) Traditional model species such as *Arabidopsis thaliana* have a short lifecycle, small size and small genome. (b) Cereal crops such as wheat have a long lifecycle, large size and a large genome. (c) New approaches such as speed breeding, rapid lifecycle varieties and improved sequencing techniques enable cereal crops to have many of the characteristics of model species. Note: plants not drawn to scale.

Mini-Maize (60 d) (McCaw *et al.*, 2016) and rice 'Xiaowei-*Se5*' (46 d) (Hu *et al.*, 2018). These rapid lifecycle cereal varieties are also much smaller than conventional varieties (Fig. 1) and are well-suited to cultivation in the controlled environment conditions used widely by plant science researchers. Although a variety of agronomically relevant traits such as disease resistance and flowering time can be studied using speed breeding or rapid lifecycle varieties (Watson *et al.*, 2018), there are certain traits, such as yield or plant height, for which research may prove difficult (Hu *et al.*, 2018), particularly in rapid lifecycle varieties.

Many of the resources which make working with Arabidopsis attractive are now available for cereal crops. Rice, maize and wheat have accurate gene model annotations, extensive molecular biology methods and sequenced mutant populations (Table 1) that are suitable for reverse genetics approaches (Settles *et al.*, 2007; Williams-Carrier *et al.*, 2010; Krasileva *et al.*, 2017; Li *et al.*, 2017). Gene expression atlases are also available for all three species (Borrill *et al.*, 2016; Xia *et al.*, 2017; Ramirez-Gonzalez *et al.*, 2018; Hoopes *et al.*, 2019). Together these resources enable routine study of gene function in rice, maize and wheat.

V. The future of cereal research

Despite the availability of new approaches and resources, one area that remains a challenge is the production of transgenic plants, especially for wheat and maize. This presents a rate-limiting step for biological understanding of gene function. Whilst transformation of rice is routinely carried out by many research groups using callus induction methods (Hiei *et al.*, 2014), maize and wheat transformation require high-quality facilities and technical expertise to transform immature embryos (Fig. 2a). This restricts transformation to specialised laboratories in universities, institutes and multi-national seed companies. A further challenge for cereals is that efficient transformation is genotype-dependent, and therefore only a handful of varieties can be transformed at high enough efficiencies to make transformation routine.

Recently, however, several methods have shown promise as means by which to expand the number of transformable varieties. In maize, the expression of the transcription factors *Baby boom* and *Wuschel2* increased both the transformation efficiency and the range of varieties transformed (Fig. 2b; Lowe *et al.*, 2016). This system also increased the transformation efficiency of rice (Lowe *et al.*, 2016) and has been proposed as a method of increasing wheat transformation efficiency and allowing genotype-independent transformation (Borrill *et al.*, 2019b). It may also be possible to extend the range of transformable genotypes by using bacteria other than *Agrobacterium tumefaciens* for transformation (Fig. 2a). For example, *Ensifer adhaerens*, a soil-related bacterium, has been shown to transform IR64, an indica rice variety that is difficult to transform using *A. tumefaciens* (Zuniga-Soto *et al.*, 2015). However, further work is required to increase the low transformation efficiencies achieved with non-Agrobacterial species and test this approach in wheat and maize. The ability to carry out transformation on any genotype would accelerate the integration of transgenic events in cereal breeding pipelines and facilitate the evaluation of the effects of the transgenic or gene editing event in a locally adapted variety.

An alternative method of altering gene function in elite lines is the use of CRISPR-Cas9 in conjunction with haploid induction to induce edits without any transformation event in the recipient plant. Kelliher *et al.* (2019) crossed a CRISPR-Cas9 expressing maize line to wheat, which produced a haploid wheat line edited at

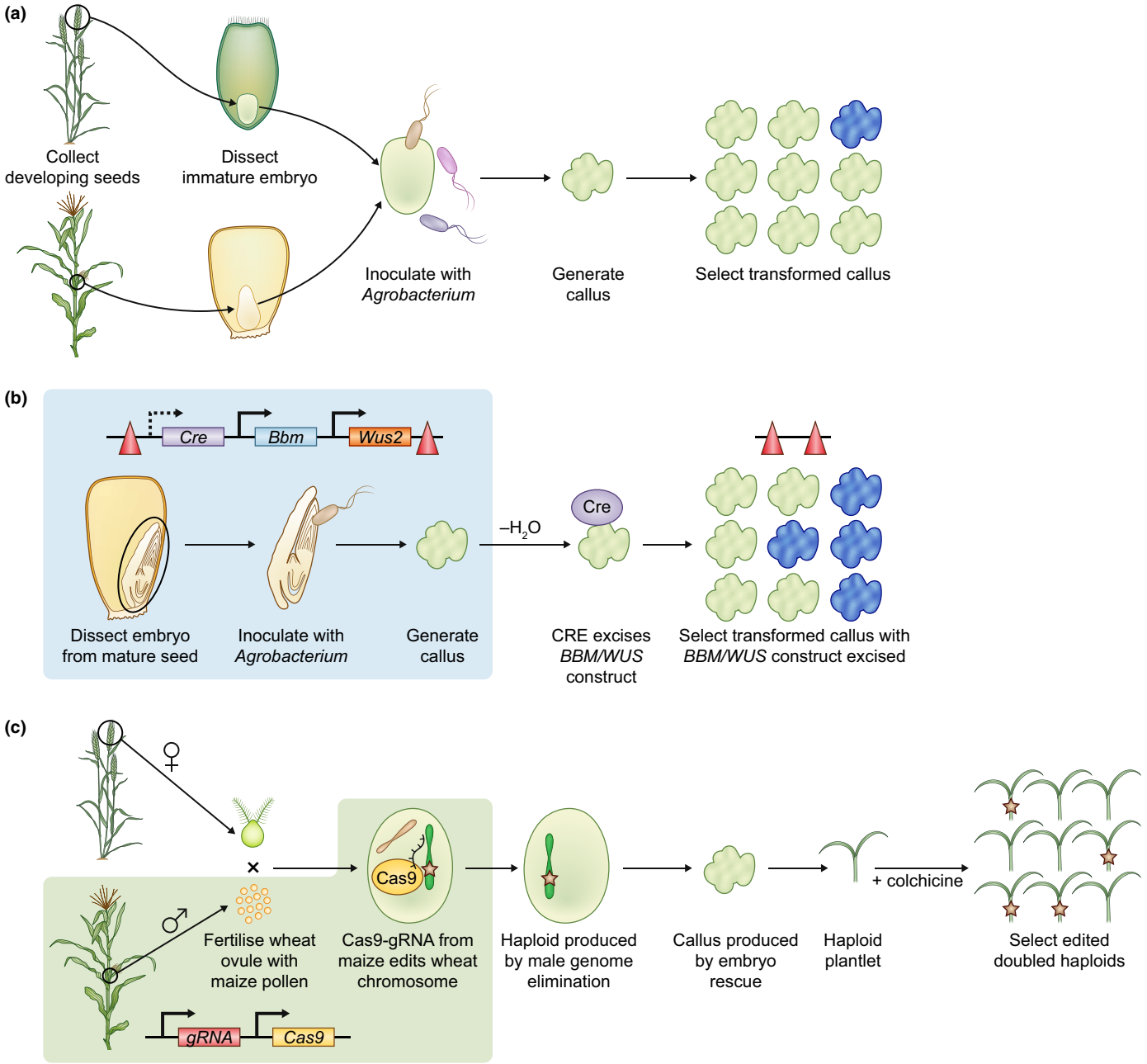

Fig. 2 Improving transformation in maize and wheat to alleviate genotype dependency. (a) Conventional transformation of maize and wheat. Immature embryos are dissected from developing seeds. The immature embryo is then inoculated with *Agrobacterium tumefaciens* (brown). A callus is generated by tissue culture and the transformed callus (blue) can be selected using a marker gene. Alternative bacterial species (pink and purple) have been proposed as one route by which to overcome genotype-dependency in transformation. (b) Expression of *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*) increases transformation efficiency and the number of genotypes which can be transformed in maize. Using transgenic maize expressing *Bbm* and *Wus2* enables embryos dissected from mature seeds to be used for transformation. The dissected embryo is inoculated with *A. tumefaciens* and undergoes tissue culture. The callus produced is desiccated to induce the expression of *Cre recombinase* (*Cre*) from a desiccation inducible promoter. Cre excises the transgenic cassette at the LoxP sites (triangles) to prevent the expression of *Bbm*/*Wus2* from causing undesirable phenotypic effects in subsequent generations. The transformed callus (blue) with the *Bbm*/*Wus2* construct excised can then be selected. The light blue background indicates stages at which the *Bbm*/*Wus2* construct is expressed. (c) Haploid inducer maize lines expressing a CRISPR/Cas9 construct can edit wheat target genes, thus bypassing a wheat transformation step. Pollen from a haploid inducer maize line expressing Cas9 with a gRNA for a wheat target gene is used to fertilize an emasculated wheat ear. The Cas9-gRNA edits the wheat target gene (brown star) in the fertilized ovule. Subsequently the male (maize) genome is eliminated to produce a haploid gene-edited embryo. The embryo is rescued by tissue culture to produce a haploid plantlet. Colchicine treatment induces chromosome doubling and gene-edited doubled haploid wheat plants can be selected. This method could be used to edit genes in a wide range of wheat varieties because it does not depend on *A. tumefaciens* mediated transformation of wheat. The green background indicates stages at which the Cas9-gRNA construct is expressed.

the target site (Fig. 2c). Although the reported efficiencies were low, the optimisation of this method presents an attractive route via which to induce edits in multiple elite wheat varieties after only one transformation event, without requiring transformation of the elite varieties themselves and with no risk of inheritance of the transgenic cassette.

CRISRP-Cas9 has also been used to rapidly domesticate orphan crops by removing undesirable traits (Lemmon *et al.*, 2018). This approach of targeting known phenotypic genes could be used to make maize, rice and wheat more amenable to laboratory studies, for example by targeting genes for reduced size. Transformation methods that can be applied to a wider range of varieties (Fig. 2) may bypass some of the limitations of the current rapid lifecycle varieties that are only available in a few genetic backgrounds. Secondly, this approach could be applied to facilitate the study of other cereals or to generate custom model species or varieties for the examination of particular traits.

The difficulties in molecular biology techniques such as transformation, which are variety dependent, show that there is still a lot to learn about the influence of genetic variation on the biology of a plant. Genomic studies are now revealing the huge range of genetic variation within plant species; for example, the re-sequencing of 3000 rice varieties has identified over 10 000 novel full-length protein-coding genes (Wang *et al.*, 2018). Some of these genes, which are absent from the reference variety Nipponbare, may have agronomically relevant functions, as has been shown for the *SUB1A* gene, which confers resistance to flooding but is not present in japonica varieties (Xu *et al.*, 2006). Pan-genome projects for the study of genomic diversity have also been initiated in maize (Brohammer *et al.*, 2018) and wheat (Borrill *et al.*, 2019b), with early results from maize underlining the importance of transposable elements in driving genomic variation (Anderson *et al.*, 2019). Leveraging the variation in these species will be critical to furthering our understanding of cereal biology and developing improved elite varieties.

Looking to the future, at the global political level there is an increased interest in food security, which will have an important influence on cereal research. In 2015 the United Nations set a Sustainable Development Goal to end hunger by 2030. This global interest has already started to increase the amount of funding allocated to cereal research, which in turn has helped to accelerate the development of many new technologies. However, it remains difficult to determine which came first – the technology or the funding. More researchers are being attracted to work on cereals, in part due to the funding landscape, but also due to the increased tractability of cereals for molecular biology work. To give one area of research as an example, cloning the first disease resistance genes in cereals took a minimum of 5–10 yr. Now with genomics approaches, resistance genes can be cloned far more rapidly, as evidenced by the exponentially increasing number of cloned resistance genes in wheat (Keller *et al.*, 2018) and papers publishing multiple resistance genes simultaneously, which would have been unthinkable even a few years ago (Steuernagel *et al.*, 2016; Marchal *et al.*, 2018). Through the techniques and resources available now, and those currently under development, it is likely that there will be similar expansions in understanding the molecular mechanisms of a whole range of traits in cereals.

VI. Conclusions

We now have the tools, resources and approaches with which to accelerate our fundamental understanding of the biology of the three major cereal crops using the species themselves, whilst considering the insights gained in traditional model systems such as Arabidopsis. Studying cereals directly will translate the promise of genetic solutions more rapidly into the field, through breeding programmes and agronomic practices. We will need to take advantage of multiple approaches to feed the growing world population and to stabilise, let alone increase, yields under climate change. Traditional model species can give us insight into conserved processes, but the opportunities to work directly on cereal crops are now too great to ignore.

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